Remarks

By way of the foregoing amendment, Claims 29, 30, 35 and 44-73 are currently pending. Claims 30, 57 and 62 have been withdrawn by the Examiner as being drawn to nonelected inventions. Claims 29, 58, and 60 have been amended without prejudice to more clearly define the invention. Claims 1-28, 31-34 and 36-43 have been previously cancelled. Claims 72-73 have been newly added. Support for these amendments can be found throughout the specification as originally filed, at least, for example, at page 5, line 37 to page 6, line 1; page 8, lines 1-4; page 11, lines 12-15; page 12, lines 1-6; and page 13, lines 19-22. No new matter enters by way of these amendments.

1. Request for Continued Examination

Further to the Request for Continued Examination filed by Applicants on July 7, 2009, claims 29, 35, 44-56, 58-61 and 63-71 were examined.

2. Withdrawn Claim Rejection

Applicants thank the Examiner for withdrawing the rejection of claims 29, 35, 44-56 and 58-61 under 35 U.S.C. § 102(e) over Sale *et al.* (U.S. Patent Application Publication No.: 2005/0026246).

3. New Claim Rejections

A. 35 U.S.C. § 112, Second Paragraph

Claims 65, 66, 69 and 70 stand rejected under 35 U.S.C. § 112, second paragraph, as allegedly being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. Applicants disagree with the Examiner's rejection.

The Examiner alleges that "[c]laims 65 and 66 are unclear in their recitation of 'hypermutation is at a rate above an order of 10^{-9} to 10^{-10} bp^{-1} $generation^{-1}$.' Claims 69 and 70 are unclear in their recitation of 'hypermutation… is at least ten times higher.'" Office Action at

page 3. The Examiner concludes that "[t]he claims fail to set an upper limit for the rate of hypermutation, thus failing to define the metes and bounds of the claims." *Id.*

The law merely requires that "the claims read in light of the specification reasonably apprise those skilled in the art of the scope ...." *Credle v. Bond*, 25 F.3d 1566, 1576, 30 U.S.P.Q.2d 1911, 1919 (Fed. Cir. 1994) (emphasis added). The degree of precision need only be "as accurate as the subject matter permits." *See Orthokinectics, Inc. v. Safety Travel Chains, Inc.* 806 F.2d 1565, 1 U.S.P.Q.2d 1081, 1088 (Fed. Cir. 1986). Accordingly, the recited rates are not impermissibly indefinite.

Applicants respectfully submit that one of ordinary skill in the art would recognize that the upper limit for the rate of hypermutation is 1, *i.e.*, every base is mutated with every cell division. The mutation rate recited in the claims is always below 1 and will never reach 1. It is not necessary to explicitly state the upper limit because a person of ordinary skill in the art will readily understand that the rate of hypermutation is always below 1. As such, Applicants submit that the Examiner's rejection is in error and should be withdrawn.

B. 35 U.S.C. § 112, New Matter

Claims 69 and 70 stand rejected under 35 U.S.C. § 112, first paragraph, as allegedly containing subject matter that was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventors, at the time the application was filed, had possession of the claimed invention. The Examiner alleges that the instant specification is devoid of description for "wherein the rate of hypermutation in said genetically modified lymphoid cell is at least ten times higher than the mutation rate in said lymphoid cell." *See* Office Action at page 3. Applicants disagree.

The law does not require *ipsis verbis* support. If a skilled artisan would have understood the inventor to be in possession of the claimed invention at the time of filing, even if every nuance of the claims is not explicitly described in the specification, then the adequate description requirement is met. MPEP § 2163; See also *Capon v. Eshhar*, 418 F.3d 1349, 1357, 76 USPQ2d 1078, 1085 (Fed. Cir. 2005)("The 'written description' requirement must be applied in the context of the particular invention and the state of the knowledge.")

One of ordinary skill in the art would be aware that the rate of mutation is at least ten times higher under hypermutation than gene conversion. In light of the specification, one of ordinary skill in the art would realize that the inventors were aware at the time of filing that the rate of mutation in a lymphoid cell capable of gene conversion was at least ten times less than the rate of hypermutation in a genetically modified lymphoid cell. Applicants submit that the limitation "wherein the rate of hypermutation in said genetically modified lymphoid cell is at least ten times higher than the mutation rate in said lymphoid cell" is directly derivable from the specification as filed, at least, for example, at page 8, lines 12-18. As such, Applicants submit that the Examiner's rejection should be withdrawn.

C. 35 U.S.C. § 112, Written Description

Claims 29, 35, 44-56, 58-61 and 63-71 stand rejected under 35 U.S.C. § 112, first paragraph, as allegedly failing to comply with the written description requirement. The Examiner alleges that "[t]he specification discloses only one lymphoid cell (chicken DT40 $AID^R\psi V^-$), that contains no mutations in RAD51 or its analogs, and replaces gene conversion by hypermutation... The specification is silent however on any other genetically modified variants of lymphoid cells from any species of animals, or a DT40 or similar cell that has a hypermutation rate higher than the rate of its non-genetically modified counterpart that contains ψV donors." Office Action at page 5. Applicants disagree.

"The descriptive text needed to meet [the written description requirement] varies with the nature and scope of the invention at issue, and with the scientific and technologic knowledge already in existence." *Capon v. Eshhar*, 418 F.3d 1349, 1357 (Fed. Cir. 2005). As acknowledged by the Examiner, Applicants demonstrate proof of concept with chicken cell DT40 $AID^R\psi V^-$. *See* Specification at Example 1, page 13, line 37 through page 19, line 17. Following that, Applicants disclose a method of producing a genetically modified lymphoid cell capable of directed and selective genetic diversification of a transgenic target nucleic acid sequence by hypermutation without deleting the endogenous ψV genes. *See Id.* at Example 2, page 19, line 25 to page 20, line 12. In particular, an Ig VJ replacement vector, pVjRepBsr (discussed below), is described for such a method. *Id.* at page 19, lines 29-30 and Figure 7A.

In *Falko-Gunter Falkner v. Inglis*, the Federal Circuit encountered analogous facts. 448 F.3d 1357 (Fed. Cir. 2006). In *Falkner*, at issue was whether the Inglis applications adequately described and enabled a poxvirus-based vaccine despite the lack of a detailed example using poxvirus. *Id.* at 1364. The Inglis patent application included a detailed example of an embodiment from a different virus than the claimed virus. The *Falkner* court held that the Inglis specification contained sufficient written description without a detailed example specific to the claimed embodiment where a person of ordinary skill in the art could apply the lessons from the detailed example to the claimed embodiment. "[A]n actual reduction to practice is unnecessary to satisfy the written description requirement." *Id.* at 1388.

In the same vein, Applicants have disclosed a method of producing a genetically modified lymphoid cell capable of directed and selective genetic diversification of a transgenic target nucleic acid sequence by hypermutation with or without endogenous ψV genes. *See* Specification, for example, at page 13, lines 19-22; Example 2 at page 19, line 25 to page 20, line 12. For example, pVjRepBsr (shown in Figure 7A) is an Ig VJ replacement vector that allows one to replace the Ig light chain VJ gene with any transgenic target nucleic acid sequence. Figure 7B shows as an example that the transgene GFP can be inserted into a lymphoid cell, causing it to be a genetically modified lymphoid cell and resulting in hypermutation of GFP regardless of the presence of endogenous ψV genes. If present, the endogenous ψV genes still do not result in gene conversion of the transgene, in this case GFP, because the transgene is a foreign gene to the lymphoid cell and therefore foreign to the endogenous ψV genes. Hypermutation of any transgene inserted into the immunoglobulin locus of a lymphoid cell occurs where the ψV genes are not capable of acting as gene conversion donors because they do not have enough homology to the transgene. Accordingly, the pVjRepBsr is an example of a vector that can be used to produce a genetically modified lymphoid cell capable of directed and selective genetic diversification of a transgenic target nucleic acid sequence by hypermutation without deleting the endogenous ψV genes. As such, the present specification provides sufficient description for claims 29, 35, 44-56, 58-61 and 63-71.

The specification describes the difference in result using pVjRepBsr (Figure 7B) with the result after stepwise targeted integration with adjacent gene conversion donors (Figure 7D).

pPseudoRepBsr (shown in Figure 7C) allows one to replace the endogenous ψV loci with one or more transgenic adjacent donor sequences capable of acting as gene conversion donors, *i.e.*, where they have homology to the transgene. *Id.* at page 20, lines 1-3. Figure 7D exemplifies that if GFP is inserted into the immunoglobulin locus of a lymphoid cell and homologous sequences (pseudogenes), in this case a ψGFP gene and its variants, are placed adjacent to the target transgene, gene conversion will occur between the pseudogenes and the transgene target. *Id.* at page 19, line 32 to page 20, line 6. In one aspect for ease of monitoring the mutation rate of the genetically modified lymphoid cell, GFP with a frameshift can be used as the transgenic target nucleic acid sequence to more easily determine when a mutation occurs that results in fluorescence. *Id.* at page 20, lines 3-6.

The instant specification states that gene conversion requires a gene conversion donor and in the absence of a gene conversion donor, the sequence in the immunoglobulin locus will undergo hypermutation. *See* Specification at page 13, lines 19-21. In the endogenous situation, gene conversion occurs because the sequences adjacent to the immunoglobulin locus are homologous. The specification states that "partial or complete deletion of the pseudo-V-genes in a cell line which continues gene conversion in cell culture leads to the activation of hypermutation in the immunoglobulin locus. Deletion of all pseudogenes results in the abolishment of gene conversion and simultaneous activation of high rates of hypermutation." *See* specification at page 8, lines 34-37. This describes obtaining hypermutation of the endogenous immunoglobulin sequence. The deletion of endogenous ψV genes is only required where homology exists between the ψV genes and the sequence in the immunoglobulin locus of the lymphoid cell. Where there is not enough homology between the sequence in the immunoglobulin locus of a genetically modified lymphoid cell (a transgene) and the endogenous ψV genes, homologous recombination cannot occur and deletion of the ψV genes is not required.

Claim 29 recites a transgenic target nucleic acid sequence in the immunoglobulin locus of a genetically modified lymphoid cell. Such a transgenic target nucleic acid sequence will not have sufficient homology to the endogenous adjacent sequences (the ψV genes) for the presence or absence of the endogenous ψV genes to significantly affect the rate of hypermutation of the recited transgenic target sequence. Claim 72 recites a transgenic target nucleic acid sequence in

the absence of an adjacent donor sequence so that no gene conversion would occur, only hypermutation, or point mutations, would occur. The endogenous ψV genes are not adjacent donor sequences for the transgenic target nucleic acid sequence because the transgenic sequence is foreign to the lymphoid cell and therefore not capable of homologous recombination with the ψV genes.

With respect to the Examiner's statement that "[t]he specification is silent however on any other genetically modified variants of lymphoid cells from any species of animals...," Applicants disagree. It is well established that claims "may be broader than the specific embodiment disclosed in a specification." *Ralston-Purina Co. v. Far-Mar Co*, 772 F.2d 1570, 1575 (Fed. Cir. 1985), *quoting In re Rasmussen*, 650 F.2d 1212, 1215 (C.C.P.A. 1981). Thus, in order for Applicants to describe a method for producing a genetically modified lymphoid cell encompassed by the claims, it is not required that every aspect of each and every method for producing a genetically modified lymphoid cell be disclosed. "It is not necessary that every permutation within a generally operable invention be effective in order for an inventor to obtain a generic claim, provided that the effect is sufficiently demonstrated to characterize a generic invention." *Capon v. Eshhar*, 418 F.3d 1349, 1359 (Fed. Cir. 2005). A person of ordinary skill in the art would, after reading the specification, recognize that Applicants had possession of the claimed subject matter.

The claimed methods can be applied to any lymphoid cell capable of gene conversion to produce a genetically modified lymphoid cell capable of directed and selective genetic diversification of a transgenic targeted nucleic acid sequence by hypermutation. The specification states that a cell used in the present invention can be any cell that is "constructed by replacing the endogenous V-gene or segments thereof with a transgene." *See* specification as filed at page 11, lines 23-24. As such, one may use any gene conversion active lymphoid cell that does not have deleterious mutations in XRCC2, XRCC3, RAD51 or their analogues as a starting material in the presently-claimed methods.

No other modifications are required to use the claimed subject matter. In particular, it is not a requirement of the current claims that the endogenous ψV genes be deleted. Example 1 provides a clear proof-of-concept that in the absence of gene conversion donors, a lymphoid cell

capable of gene conversion will diversify genes in the Ig locus by hypermutation. The experimental work described in the examples was conducted with a chicken B cell line DT40, a well-established lymphoid cell line that has gene conversion activity. The application also describes that other gene conversion-active cells such as lymphoid cells derived from chicken, cow, rabbit, sheep and pig will as well be workable for the purpose of the invention. *See*, for example, original claim 6 and page 4, lines 4-8 and page 8, lines 28-30 of the specification as filed. Further, wild-type cells are normally homologous recombination proficient and contain no mutations in the recited recombination genes. Thus, one of ordinary skill in the art would recognize that any wild-type gene conversion-active cell should be suitable for use in the claimed method.

In light of at least the above, Applicants submit that the specification as filed provides sufficient written description for the claimed subject matter. As such, the Examiner's rejection of claims 29, 35, 44-56, 58-61 and 63-71 under 35 U.S.C. § 112, first paragraph, written description, should be withdrawn.

D. 35 U.S.C. § 112, Enablement

Claims 29, 35, 44-56, 58-61 and 63-71 stand rejected under 35 U.S.C. § 112, first paragraph, as allegedly failing to comply with the enablement requirement. Applicants thank the Examiner for acknowledging that the specification is "enabling for a method for selective genetic diversification of a transgenic target nucleic acid sequence by hypermutation, in a lymphoid cell lacking ψV donors, wherein the lymphoid cell lacking ψV donors is capable of gene conversion prior to deletion of ψV donor sequences, said method comprising introducing a genetic construct comprising said target nucleic acid into the immunoglobulin locus of the lymphoid cell lacking ψV donors, whereby said target nucleic acid sequence is modified by hypermutation." *See* Office Action at page 8. However, Applicants disagree with the Examiner's statement that the specification "does not reasonably provide enablement for a method for producing a genetically modified lymphoid cell containing no deleterious mutations in genes encoding RAD51 or its analogs, capable of a hypermutation rate higher than the rate of mutation in a precursor lymphoid cell." *Id.*

At the outset, in order to establish a proper rejection, the examiner has the initial burden to establish a reasonable basis to question the enablement provided for the claimed invention. *In re Wright*, 999 F.2d 1557, 1562, 27 USPQ2d 1510, 1513 (Fed. Cir. 1993) (examiner must provide a reasonable explanation as to why the scope of protection provided by a claim is not adequately enabled by the disclosure); *see also* MPEP § 2164.04. First, the Examiner has not provided any reasonable basis to support the allegation that the endogenous ψV genes must be deleted for hypermutation where the transgenic target nucleic acid has no homology to the endogenous ψV genes. Second, the Examiner has not provided a basis for the statement that "[t]he specification is silent… on any other genetically modified variants of lymphoid cells from any species of animal." *See* Office Action at page 9.

The specification as filed teaches that a transgene (GFP for example) inserted into the Ig locus of a DT40 cell is diversified by hypermutation (*see, e.g.*, Example 2 and Figure 7). The DT40 cell is a representative species of lymphoid cells capable of gene conversion that can be used in the present methods. The specification also states that hypermutation "refers to the mutation of a nucleic acid in a cell at a rate above background." *See* specification as filed at page 8, lines 12-18. The Examiner appears to disagree with the interpretation of the data and the conclusions to be made from the facts presented in the instant specification. The Examiner, however, has not presented evidence to date that disputes the truth of those facts. As such, there is no basis for the enablement rejection. See *In re Wands*, 858 F.2d 731,737, 8 USPQ2d 1400, 1404 (Fed. Cir. 1988). See also *United States v. Telectronics, Inc.*, 857 F.2d 778, 785 8 USPQ2d 1217, 1223 (Fed. Cir. 1988) ("The test of enablement is whether one reasonably skilled in the art could make or use the invention from the disclosures in the patent coupled with information known in the art without undue experimentation.")

"[I]t is unnecessary to spell out every detail of the invention in the specification; only enough must be included… to enable such a person to make and use the invention without undue experimentation." *Falkner*, 448 F.3d at 1388. One of ordinary skill in the art would recognize that, given the teaching of the specification, it is possible without undue experimentation to determine whether a method is capable of producing a genetically modified lymphoid cell capable of selective genetic diversification of a transgenic target nucleic acid sequence by

hypermutation. The Examiner has failed to provide any basis for why the endogenous ψV donors would have to be deleted after transfection of a foreign nucleic acid sequence. The endogenous ψV genes have no homology to the target transgene in the immunoglobulin locus so they cannot be adjacent donor sequences for gene conversion. If the Examiner maintains that deleting the endogenous ψV genes is required, Applicants specifically request that the Examiner provide a basis by directing Applicants to the evidence he relied on. *See* MPEP § 2164.04.

Applicants also submit herewith post-filing art that demonstrates a realization of the experiments described in Example 2 of the specification. Arakawa *et al.*, *Nucleic Acids Research* 36(1): e1, 2008, submitted as Document BK1 in the Information Disclosure Statement filed herewith. Arakawa shows that the insertion of a transgene (GFP in this case) into the ψV^+ Ig locus of a chicken B cell results in the transgene being diversified by hypermutation when endogenous ψV genes are present. *See* Arakawa at page 3, right-hand column and at Figures 1A and 4. As described in the Results section and as illustrated in Figure 1, the Ig light chain locus of DT40 was targeted with a GFP-containing knock-in vector, pHypermut1-eGFP, resulting in the GFP gene being inserted into the Ig locus (designated IgLeGFP1) (Figure 1A). *See* Arakawa at page 3, left-hand column. The portion of the locus containing the endogenous ψV genes is reported as being present in the genetically modified lymphoid cell and is designated as ψV on the right in Figure 1A. The hypermutation activity of the cell was tested and the GFP mutants were analyzed with respect to the emission spectrum and intensity after cell sorting. Figure 4 depicts that GFP variants that underwent hypermutation differed in the intensity of the emitting light. *See* Arakawa at page 7, section spanning the columns).

These experiments using the pHypermut1-eGFP vector show that the insertion of a transgene into the ψV^+ Ig locus of a lymphoid cell results in the transgene being diversified by hypermutation. As such, this is further confirmation of the instant specification that it is not necessary to delete the endogenous ψV genes to have hypermutation of a transgene.

Applicants also submit Blagodatski *et al.*, *PLOS Genetics* 5(1): e1000332, 2009, submitted as Document BL1 in the Information Disclosure Statement filed herewith. Applicants direct the Examiner's attention to Figures 1A, 1C and 1D, which illustrate that GFP inserted into the Ig locus of a lymphoid cell capable of gene conversion containing endogenous ψV genes was

diversified by hypermutation. Blagodatski again confirms the point that the deletion of endogenous ψV genes is not a requirement for transgene diversification by hypermutation and is not necessary to produce a cell according to the present claims. "A GFP transgene in DT40 rapidly accumulates mutations, when integrated at the position of the promoter of the rearranged *IgL* locus." Blagodatski at page 2, first column.

Blagodatski also describes the targeting construct $pIgL^{GFP2}$ (illustrated in Figure 1A), which does not remove endogenous ψV genes. Clones transfected with $pIgL^{GFP2}$ are reported as being hypermutation-active. *See* Blagodatski at page 2, second paragraph; and at Figure 1C and D.

The effect of endogenous ψV genes are on hypermutation activity in genetically modified lymphoid cells is specifically addressed. *See* Blagodatski at page 2, section titled "Identification of a Diversification Activator." When a 20 kb portion of DNA containing the endogenous ψV region of the *IgL* locus was deleted, the transgene was mutated at a rate reported at "only one fold lower that the medians of the ψV positive IgL^{GFP2} subclones." Blagodatski at page 2, bottom of second column. Contrary to the Examiner's speculation, the endogenous ψV region increases the rate of transgene hypermutation. Blagodatski concludes that "the ψV locus seems to exert little, if any stimulation on the hypermutation activity of the GFP2 reporter." *See* Blagodatski at page 2, bottom of second column.

In light of the above, withdrawal of the rejection of claims 29, 35, 44-56, 58-61 and 63-71 under 35 U.S.C. § 112, first paragraph, is respectfully requested.

4. Conclusion

In view of the above, each of the presently pending claims is believed to be in immediate condition for allowance. Accordingly, the Examiner is respectfully requested to withdraw the outstanding rejections of the claims, and to pass this application to issue. The Examiner is encouraged to contact the undersigned at (202) 942-6237 should any additional information be necessary for allowance.

Respectfully submitted,

David R. Marsh (Reg. Atty. No. 41,408)
Kristan L. Lansbery (Reg. Atty. No. 53,183)
Julie L. Blum (Reg. Agent No. 61,840)

Date: February 12, 2010

ARNOLD & PORTER LLP
Attn: IP Docketing Dept.
555 Twelfth Street, N.W.
Washington, D.C. 20004-1206
(202) 942-5000 telephone
(202) 942-5999 facsimile